Derivatives of the New Ring System Indolo[1,2-*c*]benzo[1,2,3]triazine with Potent Antitumor and Antimicrobial Activity

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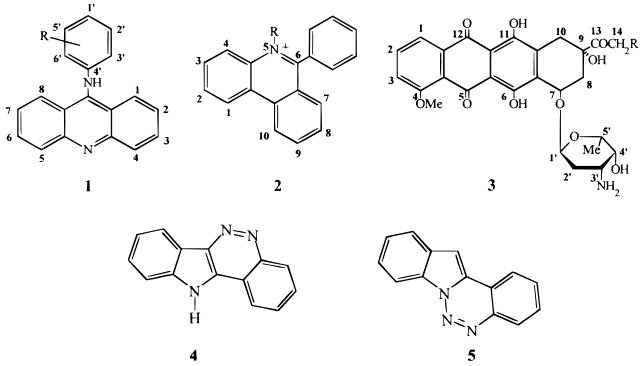
Derivatives of the new ring system indolo[1,2-*c*]benzo[1,2,3]triazine **5** were synthesized by diazotization of substituted 2-(2-aminophenyl)indoles followed by an intramolecular coupling reaction of the diazonium group with the indole nitrogen. To obtain the indolobenzotriazine system it was necessary to protect the 3 position of the indole nucleus to avoid cyclization into the indolo[3,2-*c*]cinnoline system **4**. Indolobenzotriazines **5a**−**g** were evaluated in vitro for antitumor activity against a panel of leukemia-, lymphoma-, carcinoma-, and neuroblastoma-derived cell lines. Some compounds inhibited the proliferation of T and B cell lines at submicromolar concentrations, whereas their activity against solid tumor cell lines was in the micromolar range. When evaluated for their antifungal potential **5a**,**d** inhibited some of the fungi tested, although at concentrations very close to those inhibiting the proliferation of human cells. On the contrary, all indolobenzotriazines proved fairly potent and selective inhibitors of *Streptococcus* and *Staphylococcus*. In particular **5b,c,g** were up to 80 times more potent than the reference drug streptomycin and inhibited the growth of the above Gram-positive bacteria at concentrations far lower than those cytotoxic for animal cells.

Introduction

Polycondensed nitrogen heterocycles having a planar structure can be effective pharmacophore moieties of drugs endowed with antineoplastic activity because they can intercalate into double-stranded DNA. Acridine and phenanthridine derivatives of type **1** and **2** are well-known compounds possessing such a property whose principal driving forces are stacking and charge-transfer interactions as well as hydrogen bonding and electrostatic forces.[1] In particular, it has been demonstrated that the biological activity of 9-acridinylmethanesulfonanilide derivatives **1** (R = $NHSO_2Me$) correlates with their DNA association constants, the more active compounds being those that more tightly bind to DNA.[2] Ethidium derivatives **2** (R = Et) and anthracycline antitumor antibiotics **3** also tightly bind to DNA and show a strong specificity for GC residues.[3] Doxorubicin (**3**, R = OH), in particular, interacts with the 2-amino group of guanine.[4] In addition compounds of type **1** and **3** target the topoisomerase II.[5]



1 **2** **3** **4** **5**

As a result of our interest in heterocycles annelated with either pyrrole or indole rings as potential intercalating agents, we have recently reported a new synthesis of some derivatives of the indolo[3,2-*c*]cinnoline ring system **4** and have presented evidence for their in vitro antitumor activity.[6] We now describe the synthesis of derivatives of the new ring system indolo[1,2-*c*]benzo[1,2,3]triazine **5** and report on the in vitro evaluation of their biological activity.

Chemistry

Compounds **6**, the key intermediates for the synthesis of derivatives of the indolobenzotriazine ring system, were conveniently prepared by an intramolecular Wittig reaction of suitable ylides, which were easily obtained, in turn, from 2-aminobenzyltriphenylphosphonium salts and 2-nitrobenzoyl chlorides (Scheme 1). As recently pointed out,[6] for the preparation of derivative **7f** we preferred the classical Fisher reaction due to its higher yields. This latter compound was therefore synthesized starting from 2-aminoacetophenone and 4-chlorophenylhydrazine.

To obtain the indolobenzotriazine system it was necessary to protect the 3 position of the indole ring in order to avoid the ring closure leading to the indolocinnoline system during the diazotization reaction. Since halogenation with *N*-halosuccinimides in dimethylformamide gave excellent yields in monohalopyrroles,[7] we chose this reaction to protect the 3 position of the indole nucleus. Thus, compound **6a** was brominated with NBS in dimethylformamide to give the corresponding 3-bromo-2-(2-nitrophenyl)indole (**8**) in quantitative yield. Reduction of the nitro group with iron in acetic acid led to the corresponding amino derivative **9** in 85% yield. Diazotization of the amino derivative **9** with sodium nitrite in acetic acid failed to give the expected indolo-

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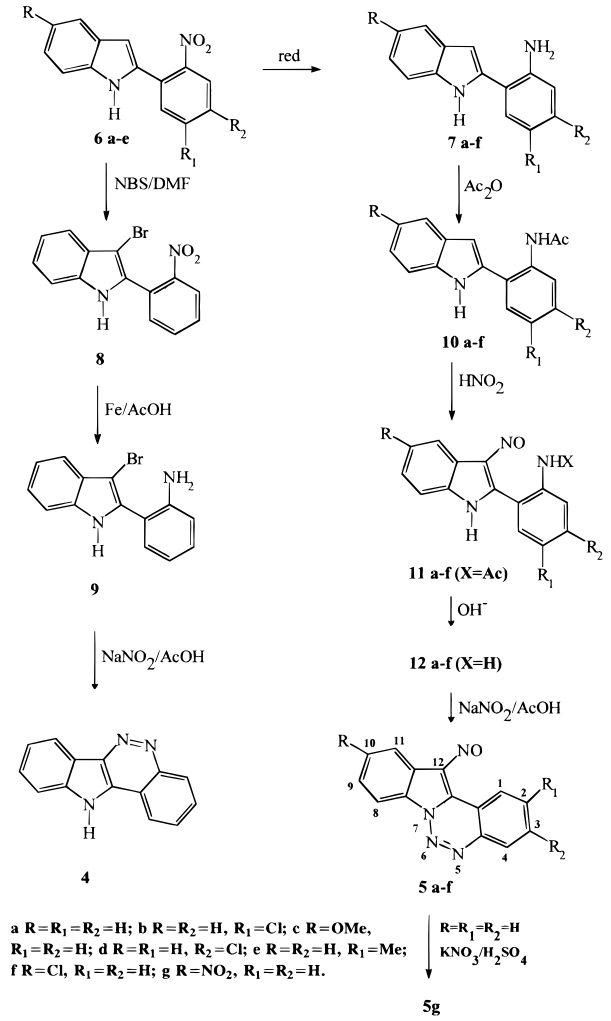
Scheme 1



[1,2-*c*]benzo[1,2,3]triazine, as a result of the intramolecular coupling reaction with the indole nitrogen, but afforded the indolo[3,2-*b*]cinnoline **4**, through an aza-dehalogenation which can be envisaged as a Japp−Klingemann reaction with extrusion of bromonium ion, as previously observed in the pyrrole series.[8] Therefore, to protect the 3 position of the indole ring, it was necessary to undertake a different approach. To this end, the nitroso group seemed the most suitable one for protecting the 3 position, due to the fact that it makes more acidic the imino proton and more reactive the ring nitrogen. Moreover, even if tautomerism to the isonitroso form (which could originate a 3*H*-indole structure) could take place, the indole nitrogen (now a pyridine-like nitrogen) was deemed capable to undergo an intramolecular coupling reaction with the diazonium group.

Thus compounds **6a,c,e** were reduced to the corresponding amino derivatives **7** with hydrogen and Palladium on charcoal, whereas compounds **6b,d** were reacted with iron and acetic acid to avoid dehalogenation. Compounds **7** were acetylated nearly quantitatively (95−99%) to give the corresponding acetylamino compounds **10** which, in turn, were nitrosated at room temperature with sodium nitrite in acetic acid to give 3-nitrosoindoles **11** in excellent yields (93−97%). Removal of the protecting group gave the amino compounds **12a**−**f**. Subsequent diazotization afforded the expected indolo[2,1-*c*]benzo[1,2,3]triazines **5a**−**f** in very high yields (80−100%). The indolobenzotriazine **5g** was obtained by direct nitration with potassium nitrate in sulfuric acid of the unsubstituted indolobenzotriazine **5a** (yield 75%).

Table 1. Antitumor Activity of Indolo[1,2-*c*]benzo[1,2,3]triazines

	IC_{50} (μM)[a]							
cell line	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	Doxo[b]
leukemia/lymphoma								
L1210	12	7.4	13	2.4	1.7	0.7	9.2	0.23
Wil2-NS	4.4	2.7	3.7	0.8	0.8	0.2	12	0.02
CCRF-SB	7.4	7.0	5.0	2.8	0.7	0.4	11	0.01
CCRF-CEM	7.0	5.0	7.2	1.8	0.5	0.08	8.2	0.1
MOLT-4	2.3	2.0	1.3	1.1	0.5	0.2	6.6	0.03
MT-4	1.4	1.7	1.1	0.9	1.3	0.5	1.2	0.02
carcinoma								
CHO-K1	45	31	>50	9.3	6.5	1.6	42	0.4
HT-29	50	24	>50	35	3.1	1.3	18	0.05
HeLa	10	5.4	11	9.2	9.5	2.7	16	0.2
ACHN	12	11	14	7.6	8.7	2.6	>50	0.4
5637	4.2	2.1	6.0	2.6	1.9	0.3	29	0.02
neuroblastoma								
IMR-32	3.3	2.3	2.3	2.2	2.8	0.8	12	0.01

[a] Compound concentration required to reduce cell multiplication by 50% under conditions allowing untreated cells to undergo at least three consecutive rounds of multiplication. Values are the mean of at least three separate experiments. Variability among triplicate samples was less than 22%. L1210, mouse leukemia; Wil2-NS, human splenic B lymphoblastoid cells; CCRF-SB, human acute B lymphoblastic leukemia; CCRF-CEM and MOLT-4, human acute T lymphoblastic leukemia; MT-4, human $CD4^+$ T cells expressing the TAT gene of HTLV-1; CHO-K1, Chinese hamster ovary; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma; 5637, human bladder carcinoma; IMR-32, human neuroblastoma. [b] Doxo, doxorubicin (**3**, R = OH).

Biology

Title compounds were evaluated in vitro for antitumor, antiviral, antifungal, and antibacterial activity.

Antitumor Activity. The antitumor activity of indolobenzotriazines **5a**−**g** was evaluated against a panel of leukemia-, lymphoma-, carcinoma-, and neuroblastoma-derived cell lines in comparison with the reference drug doxorubicin (Doxo) (Table 1). Although less potent than Doxo, test compounds inhibited the proliferation of B and T leukemic cell lines at submicromolar/micromolar concentrations. The most potent indolobenzotriazine derivative was **5f** (IC_{50} range = 0.08−0.7 μM) followed, in order of decreasing potency, by **5e**,**d** (IC_{50} range = 0.5−2.8 μM) and by **5b**,**c**,**a**,**g** (IC_{50} range = 1.1−13 μM). The antiproliferative activity of test compounds against cell lines derived from solid tumors was again lower than that of Doxo, but still significant, in particular in the case of **5f** (IC_{50} range = 0.3−2.7 μM) and **5e** (IC_{50} range = 1.9−9.5 μM).

Antiproliferative Activity against Drug-Resistant Tumor Cell Lines. Drug resistance is a relevant therapeutic problem caused by the emergence of tumor cells possessing different mechanisms which confer resistance to a variety of anticancer drugs. Among the more common mechanisms are those related to the overexpression of glycoproteins capable to mediate the efflux of different drugs such as Doxo, vincristine, and etoposide or to altered contents of target enzymes (topoisomerases I and II). Therefore, it was interesting to investigate whether **5f** was inhibitory to drug-

Table 2. Effect of **5f** on the Proliferation of Wild-Type and Drug-Resistant KB Cells

	IC_{50} (μM)[a]				
compd	KB_{wt}	KB^{MDR}	KB^{V20C}	KB^{7D}	KB^{Camp}
5f	1.8 ± 0.28	1.1 ± 0.17	2.1 ± 0.3	1.7 ± 0.4	1.9 ± 0.25
doxorubicin	0.04 ± 0.015	0.50 ± 0.14	0.4 ± 0.08	0.65 ± 0.2	0.06 ± 0.007
vincristine	0.001 ± 0.0005	0.35 ± 0.05	0.05 ± 0.018	0.035 ± 0.01	0.005 ± 0.001
etoposide	1.45 ± 0.9	26.2 ± 9.6	>50	>50	1.30
camptothecin	0.04 ± 0.005	0.07 ± 0.14	0.03 ± 0.003	0.035 ± 0.005	1.04

[a] Compound concentration required to reduce cell multiplication by 50% under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (±SD) for three independent determinations.

Table 3. Cytotoxicity of Indolo[1,2-*c*]benzo[1,2,3]triazines for Normal Human Lymphocytes

	CC_{50} (μM)[a]		
cell line	**5e**	**5f**	Doxo[b]
PBL_{PHA}[c]	0.5 (±0.19)	0.2 (±0.08)	0.04 (±0.01)
$PBL_{resting}$[d]	0.6 (±0.25)	0.9 (±0.25)	0.09 (±0.03)
leukemia[e]	0.9	0.35	0.06

[a] Compound concentration required to reduce cell multiplication by 50%. Values are the mean (±SD) for three separate experiments. [b] Doxo, doxorubicin (**3**, R = OH). [c] PBL were stimulated with PHA and then resuspended in IL2-containing medium in the presence of drugs. [d] PBL were treated with test drugs for 3 days, then stimulated with PHA, and resuspended in drug-free medium. [e] Data are the mean IC_{50} values obtained with lymphoblastoid cell lines.

resistant cell lines. Thus, we evaluated its activity against the following KB subclones (Table 2):

1. KB^{MDR}, obtained by infection of KB_{wt} with a retroviral vector carrying the human *mdr-1* gene and maintained under continuous treatment with Doxo. These cells express a membrane glycoprotein (Pgp) which is responsible for the efflux of many unrelated drugs (hence the name MDR, or multidrug resistance). 2. KB^{V20C}, selected under continuous treatment with vincristine. These cells possess an MDR phenotype related to the overexpression of the *mdr-1* gene and, like the KB^{7D} subclone, mediate the efflux of Doxo, vincristine, and etoposide but are sensitive to camptothecin. 3. KB^{Etop}, selected under continuous treatment with etoposide, a topoisomerase II inhibitor largely used in the clinic. In this case resistance is due to overexpression of the *mrp* gene, which codes for a membrane glycoprotein (MRP) capable of mediating the efflux of etoposide, Doxo, and vincristine. These cells also express altered levels of topoisomerase II. 4. KB^{Camp}, selected under continuous treatment with camptothecin, a topoisomerase I inhibitor. These cells are characterized by a low expression of topoisomerase I and increased expression of topoisomerase II.

Interestingly, **5f** proved fully inhibitory to all these resistant cell lines, thus suggesting that it neither is subject to the pump mediating the efflux of many antitumor drugs nor interferes with the DNA synthesis by affecting the topoisomerase I and II-catalyzed step.

Cytotoxicity for Normal Cells. To obtain more insights into the cytotoxic potential of test compounds for normal human cells, the two indolobenzotriazines endowed with the most potent antiproliferative activity were assayed in vitro against peripheral blood lymphocytes (PBL) from healthy donors. Doxo was run as reference drug (Table 3). **5e**,**f** proved cytotoxic for both PHA-stimulated and resting PBL at concentrations close to those active against lymphoblastoid cell lines. In this respect, however, they did not differ from Doxo, which also proved equally cytotoxic for proliferating and resting normal PBL and leukemic cell lines.

Antiviral Activity. When indolobenzotriazines were tested against human immunodeficency virus type-1 (HIV-1) in de novo infected cells, they resulted ineffective at concentrations close to those cytotoxic for uninfected MT-4 cells (results not shown). Title compounds were also inactive against other DNA and RNA viruses such as herpes simplex type-1, coxsackie, and vesicular stomatitis viruses (results not shown).

Antimycotic Activity. Test compounds were then evaluated for antifungal activity in comparison with miconazole as reference drug (Table 4). Compounds **5c**,**e**,**f** were totally devoid of antimycotic activity up to 200 μM, whereas the other derivatives were inhibitory to some of the fungi tested. The most sensitive species was *Cryptococcus neoformans*, one of the agents of opportunistic infections in AIDS patients, against which **5a,b,d** showed potencies comparable to that of miconazole. However, the fact that test compounds inhibited the fungal growth at concentrations that were in the same order of magnitude of those cytotoxic for human cells makes **5a**,**b**,**d**,**g** antifungal agents endowed with a very poor selectivity index (ratio CC_{50}/MIC). It is worth noting, however, that indolobenzotriazines **5e**,**f**, which showed the most potent antitumor activity in vitro, like Doxo, lacked antimycotic activity.

Antibacterial Activity. The antibacterial potential of indolobenzotriazines was evaluated against representatives of Gram-negative, Gram-positive, and *Mycobacteria* (Table 5) together with that of streptomycin, used as reference drug. In general, title compounds were inactive or only marginally active against *Salmonella*, *Shigella*, *M. fortuitum*, and *M. smegmatis*. By contrast, all compounds were inhibitory to *Streptococcus* and *Staphylococcus*, against which some derivatives (**5b**,**c**,**g**) proved both potent and selective, being active at concentrations up to 80 times lower than those effective against human cells. Noteworthy **5b**,**c**,**g** were also up to 80-fold more potent than streptomycin against *Streptococcus* and *Staphylococcus* and showed the same potency against penicillin-resistant strains of *S. aureus* (data not shown).

SAR Studies. SAR studies indicate that maximum antitumor activity in vitro correlates with the presence of either a chlorine atom at position 10 (**5f**) or a methyl group at position 2 (**5e**), whereas the absence of substituents at positions 10 and 2 (**5a**), the substitution of a chlorine atom for a methoxy (**5c**) or nitro (**5g**) group at position 10, and the substitution of a methyl group for a chlorine atom (**5b**) at position 2 significantly decrease the activity. Moving the chlorine atom from position 2 to position 3 (**5d**) partially restores the antitumor activity in vitro.

Table 4. Antifungal Activity of Indolo[1,2-*c*]benzo[1,2,3]triazines

	MIC (μM)[a]								
species	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	Doxo[b]	Mic[c]
C. albicans	19	150	>200	12	>200	>200	66	>2.5	7.5
C. paratropicalis	19	150	>200	25	>200	>200	22	>2.5	0.9
C. parapsilosis	19	19	>200	3.0	>200	>200	66	>2.5	7.5
C. neoformans	3.1	0.8	>200	1.6	>200	>200	7.4	>2.5	0.9
T. mentagrophytes	9.4	9.4	>200	50	>200	>200	66	>2.5	0.9
A. fumigatus	19	9.4	>200	6.2	>200	>200	>200	>2.5	1.9

[a] Minimum inhibitory concentration. Values are the mean of at least three separate experiments. Variability among triplicate samples was less than 15%. [b] Doxo, doxorubicin (**3**, R = OH). [c] Mic, miconazole.

Table 5. Antibacterial Activity of Indolo[1,2-*c*]benzo[1,2,3]triazines

	MIC (μM)[a]							
species	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	Strep[b]
Salmonella	25	>200	>200	>200	>200	>200	66	8.6
Shigella	25	>200	>200	>200	>200	>200	22	2.1
Streptococcus	0.8	0.1	0.1	0.4	0.8	0.8	0.09	2.1
Staphylococcus	0.8	0.05	0.2	0.4	0.4	0.4	0.09	4.2
M. fortuitum	>5	>5	>5	>5	>5	>5	>5	4.0
M. smegmatis	2.5	4.2	>5	>5	>5	>5	ND	5.0
MAC	1.2	2.5	2.5	0.6	5	0.6	ND	7.4
M. tuberculosis (ATCC)	4.6	1.8	5.3	4.4	9.3	4.7	ND	5.4
M. tuberculosis (clinical isolate 1104)	7.4	1.6	4.5	5.9	9.2	≥10	ND	2.9

[a] Minimum inhibitory concentration. Values are the mean of at least three separate experiments. Variability among triplicate samples was less than 18%. [b] Strep, streptomycin.

It is worth noting that maximum potency of antifungal activity correlates with the absence of substituents (**5a**) or the presence of a chlorine atom at position 3 (**5d**). **5d** is the sole compound capable of potently inhibiting the proliferation of both animal and fungal cells, whereas the derivatives endowed with the highest antitumor activity in vitro (**5e**,**f**) are totally ineffective on fungal growth. On the other hand, potent and selective antibacterial activity correlates with the presence of a chlorine atom at position 2 (**5b**) or with a methoxy (**5c**) or nitro (**5g**) group at position 10. Interestingly, since both **5c**,**g** are ineffective as antifungal agents and are poorly cytotoxic against human cell lines, they can be considered as antibacterial agents endowed with a certain degree of selectivity.

Conclusions

The data reported herein indicate that indolobenzotriazines are endowed with a broad spectrum of biological activities, ranging from antitumor to antifungal and antibacterial. Interestingly, their spectrum and potency can be modulated by introducing certain substituents at different positions of the benzene moieties, and this allows to obtain compounds which show activities specific for different cell types. Therefore, whereas compounds **5e**,**f** are endowed with potent antitumor activity, compounds **5a**,**d** are the most potent antifungal agents, and derivatives **5b,c,g** are the most potent against bacteria.

On the basis of these results, experiments aimed at defining the targets and the mechanisms of the inhibitory effect against eukaryotic and prokaryotic cells are in progress.

Experimental Section

(A) Chemistry. All melting points were taken on a Buchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 solutions (TMS as internal reference) at 200 and 50.3 MHz, respectively, using a Bruker AC series 200-MHz spectrometer. Column chromatography was performed with Merck silica gel, 230−400 mesh ASTM. Elemental analyses were within ±0.4% of the theoretical values.

Synthesis of Substituted 2-(2-Nitroaryl)indoles 6a−e. Compounds **6a−e** were prepared by intramolecular Wittig reaction of 2-aroylamidobenzyltriphenylphosphonium salts according to the procedure previously described for **6a−d**.[6] 2-(5-Methyl-2-nitrophenyl)indole (**6e**) was purified by column chromatography (eluant: toluene−diethyl ether, 9:1): yield 75%; mp 76 °C; IR 3400 (NH) cm^{-1}; ^{1}H NMR δ 2.45 (3H, s, CH_3), 6.53 (1H, d, J = 1.5 Hz, H-3), 7.03 (1H, dt, J = 7.3 Hz, J = 1.0 Hz, H-5), 7.15 (1H, dt, J = 7.3 Hz, J = 1.0 Hz, H-6), 7.42 (2H, dd, J = 7.3 Hz, J = 1.0 Hz, H-7 and H-4′), 7.56 (1H, dd, J = 7.3 Hz, J = 1.0 Hz, H-4), 7.61 (1H, d, J = 1.0 Hz, H-6′), 7.90 (1H, d, J = 7.3 Hz, H-3′), 10.06 (1H, bs, NH); ^{13}C NMR δ 20.8 (q), 101.4 (d), 111.5 (d), 119.4 (d), 120.4 (d), 122.0 (d), 124.3 (d), 126.5 (s), 128.1 (s), 129.3 (d), 131.8 (d), 132.9 (s), 137.0 (s), 138.4 (s), 143.3 (s). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

Synthesis of Substituted 2-(2-Aminoaryl)indoles 7a−f. Procedure A: Compounds **7a,c,e** were prepared by catalytic reduction of the corresponding nitro derivatives **6** over 10% Pd on charcoal in a Parr apparatus according to the procedure previously described for **7a,c**.[6] 2-(2-Amino-5-methylphenyl)indole (**7e**) was recrystallized from ethanol: yield 86%; mp 199−200 °C; IR 3377 and 3302 (NH_2), 3229 (NH) cm^{-1}; ^{1}H NMR δ 2.23 (3H, s, CH_3), 4.99 (2H, s, NH_2), 6.66 (1H, d, J = 2.0 Hz, H-3), 6.74 (1H, d, J = 7.8 Hz, H-3′), 6.89 (1H, dd, J = 7.8 Hz, J = 2.0 Hz, H-4′), 7.00 (1H, dt, J = 6.8 Hz, J = 2.0 Hz, H-5), 7.08 (1H, dt, J = 6.8 Hz, J = 2.0 Hz, H-6), 7.21 (1H, d, J = 2.0 Hz, H-6′), 7.40 (1H, dd, J = 6.8 Hz, J = 2.0 Hz, H-7), 7.52 (1H, dd, J = 6.8 Hz, J = 2.0 Hz, H-4), 10.36 (1H, bs, NH); ^{13}C NMR δ 20.2 (q), 99.9 (d), 111.1 (d), 116.1 (d), 117.1 (s), 119.0 (d), 119.7 (d), 121.0 (d), 124.9 (s), 128.6 (2×s), 129.0 (2×d), 136.2 (s), 143.2 (s). Anal. ($C_{15}H_{14}N_2$) C, H, N.

Procedure B: Compounds **7b,d,** bearing a halogen, were prepared by reduction with iron in acetic acid of the corresponding nitro compounds **6b,d**, according to the procedure previously described.[6]

Procedure C: 2-(2-Aminophenyl)-5-chloroindole (**7f**) was prepared by a Fisher indole synthesis refluxing for 3 h a mixture of 2′-aminoacetophenone hydrochloride (4.05 g, 30 mmol), 4-chlorophenylhydrazine hydrochloride (6.37 g, 30 mmol), and sodium acetate (3.2 g, 40.0 mmol) in ethanol (34

mL) and acetic acid (12 mL). After cooling the reaction mixture was poured onto crushed ice, and the hydrazone precipitated was filtered off, air-dried, and employed without further purification. Thus a mixture of the hydrazone (7.78 g, 30 mmol) and polyphosphoric acid (40 g) was heated at 130 °C for 10 min. After cooling at 90 °C, cold water (300 mL) was added and the suspension was neutralized with aqueous sodium hydroxide (5%). The solid was filtered off, air-dried, and recrystallized from ethanol: yield 80%; mp 148−149 °C; IR 3381 and 3308 (NH_2), 3225 (NH) cm^{-1}; ^1H NMR δ 5.23 (2H, s, NH_2), 6.68 (1H, dt, $J = 7.4$ Hz, $J = 1.2$ Hz, H-5′), 6.69 (1H, d, $J = 1.8$ Hz, H-3), 6.85 (1H, dd, $J = 7.4$ Hz, $J = 1.2$ Hz, H-3′), 7.07 (1H, dd, $J = 8.5$ Hz, $J = 1.8$ Hz, H-6), 7.08 (1H, dt, $J = 7.4$ Hz, $J = 1.2$ Hz, H-4′), 7.35 (1H, dd, $J = 7.4$ Hz, $J = 1.2$ Hz, H-6′), 7.40 (1H, d, $J = 8.5$ Hz, H-7), 7.56 (1H, d, $J = 1.8$ Hz, H-4), 11.46 (1H, bs, NH); ^{13}C NMR δ 99.7 (d), 112.5 (d), 115.9 (d), 116.5 (s), 116.6 (d), 118.8 (d), 120.9 (d), 123.6 (s), 128.8 (d), 128.9 (d), 129.8 (s), 134.8 (s), 138.0 (s), 145.8 (s). Anal. ($C_{14}H_{11}N_2Cl$) C, H, N.

Synthesis of 3-Bromo-2-(2-nitrophenyl)indole (8). To a solution of **6a** (1.2 g, 5 mmol) in anhydrous dimethylformamide (20 mL) was added a solution of *N*-bromosuccinimide (0.9 g, 5 mmol) in dimethylformamide (10 mL) dropwise at room temperature. After 2 h the reaction mixture was poured onto crushed ice; the solid precipitated was filtered off and air-dried to give **8**, which was pure enough to give satisfactory analytical and spectral data: yield 100%; mp 146 °C; IR 3368 (NH) cm^{-1}; ^1H NMR δ 7.21 (1H, dt, $J = 7.3$ Hz, $J = 1.2$ Hz, H-5), 7.30 (1H, dt, $J = 7.3$ Hz, $J = 1.2$ Hz, H-6), 7.49 (2H, dd, $J = 7.3$ Hz, $J = 1.2$ Hz, H-4 and H-7), 7.81 (2H, dt, $J = 7.5$ Hz, $J = 1.3$ Hz, H-4′ and H-5′), 7.91 (1H, dd, $J = 7.5$ Hz, $J = 1.3$ Hz, H-6′), 8.23 (1H, dd, $J = 7.5$ Hz, $J = 1.3$ Hz, H-3′), 12.02 (1H, s, NH); ^{13}C NMR δ 112.2 (d), 118.6 (d), 120.6 (d), 123.4 (d), 125.1 (d), 125.9 (s), 126.9 (s), 130.7 (d), 131.6 (s), 137.6 (d), 133.7 (d), 133.7 (s), 135.8 (s), 148.9 (s). Anal. ($C_{14}H_9N_2O_2Br$) C, H, N.

Synthesis of 2-(2-Aminophenyl)-3-bromoindole (9). A solution of **8** (0.95 g, 3 mmol) in acetic acid (30 mL) was heated at 40 °C; then iron powder (0.85 g, 15 mmol) was added over 30 min. After the addition was complete, the mixture was kept at 40 °C overnight and then poured onto crushed ice. The precipitate was collected, air-dried, and recrystallized from ethanol: yield 85%; mp 154−155 °C; IR 3414 and 3343 (NH_2), 3130 (NH) cm^{-1}; ^1H NMR δ 5.55 (2H, s, NH_2), 6.80 (1H, dd, $J = 7.5$ Hz, $J = 1.6$ Hz, H-3′), 6.88 (1H, dt, $J = 7.5$ Hz, $J = 1.6$ Hz, H-5′), 7.21−7.26 (3H, m, H-4′, H-4 and H-7), 7.34 (2H, dt, $J = 7.4$ Hz, $J = 1.6$ Hz, H-5 and H-6), 7.60 (1H, dt, $J = 7.5$ Hz, $J = 1.6$ Hz, H-6′), 8.45 (1H, s, NH); ^{13}C NMR δ 91.3 (s), 111.1 (d), 116.2 (d), 118.6 (d), 119.2 (d), 120.8 (d), 123.3 (d), 128.0 (s), 130.3 (d), 131.3 (d), 131.3 (s), 133.0 (s), 135.2 (s), 144.8 (s). Anal. ($C_{14}H_{11}N_2Br$) C, H, N.

Diazotization of 2-(2-Aminophenyl)-3-bromoindole (9). To a stirred solution of the amine **9** (0.57 g, 2 mmol) in acetic acid (30 mL) was added sodium nitrite (0.14 g, 2 mmol) dissolved in the minimum amount of water dropwise at 0−5 °C. The reaction mixture was kept at the same temperature for 3 h. It was then poured onto crushed ice and neutralized with aqueous sodium hydroxide (5%). The precipitate was filtered off, air-dried, and recrystallized from ethanol to give the indolo[3,2-*c*]cinnoline **4** (yield 70%) which had analytical and spectral data (IR, NMR) identical to an authentic sample.[6]

Synthesis of Substituted 2-(2-Acetylaminophenyl)-indoles 10a−f. 2-(2-Aminophenyl)indoles **7a−f** (7 mmol) were dissolved in acetic anhydride (30 mL) and stirred at room temperature for 2 h. The solution was then poured onto crushed ice. The solid precipitated was filtered off, air-dried, and recrystallized from ethanol.

2-(2-Acetylaminophenyl)indole (10a): yield 98%; mp 150 °C; IR 3383 (*NH*Ac), 3354 (NH), 1670 (CO) cm^{-1}; ^1H NMR δ 2.05 (3H, s, CH_3), 6.70 (1H, d, $J = 1.4$ Hz, H-3), 7.00 (1H, dt, $J = 7.0$ Hz, $J = 2.0$ Hz, H-5), 7.11 (1H, dt, $J = 7.0$ Hz, $J = 2.0$ Hz, H-6), 7.31 (2H, dt, $J = 6.3$ Hz, $J = 1.8$ Hz, H-4′ and H-5′), 7.42 (1H, dd, $J = 7.0$ Hz, $J = 2.0$ Hz, H-7), 7.56 (1H, dd, $J = 7.0$ Hz, $J = 2.0$ Hz, H-4), 7.63 (2H, dd, $J = 6.3$ Hz, $J = 1.8$ Hz, H-3′ and H-6′), 9.43 (1H, s, *NH*Ac), 11.35 (1H, bs, NH); ^{13}C NMR δ 23.4 (q), 101.3 (d), 111.2 (d), 119.1 (d), 120.0 (d), 121.3 (d), 125.3 (d), 126.6 (d), 127.0 (s), 127.6 (d), 128.3 (d), 128.9 (s), 134.8 (s), 134.9 (s), 136.5 (s), 168.6 (s). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

2-(2-Acetylamino-5-chlorophenyl)indole (10b): yield 96%; mp 213 °C; IR 3383 (*NH*Ac), 3354 (NH), 1670 (CO) cm^{-1}; ^1H NMR δ 2.04 (3H, s, CH_3), 6.77 (1H, d, $J = 1.0$ Hz, H-3), 7.00 (1H, dt, $J = 7.5$ Hz, $J = 1.6$ Hz, H-5), 7.11 (1H, dt, $J = 7.5$ Hz, $J = 1.6$ Hz, H-6), 7.37 (1H, dd, $J = 8.5$ Hz, $J = 1.8$ Hz, H-4′), 7.40 (1H, dd, $J = 7.5$ Hz, $J = 1.6$ Hz, H-7), 7.56 (1H, dd, $J = 7.5$ Hz, $J = 1.6$ Hz, H-4), 7.62 (1H, d, $J = 8.5$ Hz, H-3′), 7.67 (1H, d, $J = 1.8$ Hz, H-6′), 9.52 (1H, s, *NH*Ac), 11.45 (1H, bs, NH); ^{13}C NMR δ 23.7 (q), 102.5 (d), 111.7 (d), 119.6 (d), 120.6 (d), 122.2 (d), 127.5 (d), 128.4 (d), 128.6 (d), 128.6 (s), 129.0 (s), 129.6 (s), 133.5 (s), 134.1 (s), 136.9 (s), 169.1 (s). Anal. ($C_{16}H_{13}N_2OCl$) C, H, N.

2-(2-Acetylaminophenyl)-5-methoxyindole (10c): yield 95%; mp 197 °C; IR 3350 (*NH*Ac), 3277 (NH), 1645 (CO) cm^{-1}; ^1H NMR δ 2.06 (3H, s, CH_3), 3.76 (3H, s, OCH_3), 6.64 (1H, d, $J = 1.5$ Hz, H-3), 6.76 (1H, dd, $J = 8.7$ Hz, $J = 2.4$ Hz, H-6), 7.07 (1H, d, $J = 2.4$ Hz, H-4), 7.28 (1H, dt, $J = 8.3$ Hz, $J = 1.8$ Hz, H-5′), 7.30 (1H, d, $J = 8.7$ Hz, H-7), 7.31 (1H, dt, $J = 8.3$ Hz, $J = 1.8$ Hz, H-4′), 7.60 (1H, dd, $J = 8.3$ Hz, $J = 1.8$ Hz, H-6′), 7.63 (1H, dd, $J = 8.3$ Hz, $J = 1.8$ Hz, H-3′), 9.44 (1H, s, *NH*Ac), 11.21 (1H, bs, NH); ^{13}C NMR δ 23.8 (q), 55.5 (q), 101.6 (d), 101.7 (d), 112.1 (d), 112.3 (d), 125.6 (d), 126.9 (d), 127.4 (s), 127.8 (d), 129.1 (d), 129.1 (s), 132.0 (s), 135.1 (s), 135.7 (s), 153.8 (s), 169.0 (s). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

2-(2-Acetylamino-4-chlorophenyl)indole (10d): yield 97%; mp 215 °C; IR 3350 (*NH*Ac), 3317 (NH), 1684 (CO) cm^{-1}; ^1H NMR δ 2.08 (3H, s, CH_3), 6.73 (1H, d, $J = 1.6$ Hz, H-3), 7.02 (1H, dt, $J = 7.2$ Hz, $J = 1.4$ Hz, H-5), 7.12 (1H, dt, $J = 7.2$ Hz, $J = 1.4$ Hz, H-6), 7.32 (1H, dd, $J = 8.0$ Hz, $J = 1.6$ Hz, H-5′), 7.43 (1H, d, $J = 8.0$ Hz, H-6′), 7.57 (1H, dd, $J = 7.2$ Hz, $J = 1.4$ Hz, H-7), 7.62 (1H, dd, $J = 7.2$ Hz, $J = 1.4$ Hz, H-4), 7.82 (1H, d, $J = 1.6$ Hz, H-3′), 9.57 (1H, s, *NH*Ac), 11.43 (1H, bs, NH); ^{13}C NMR δ 23.9 (q), 102.1 (d), 111.7 (d), 119.5 (d), 120.4 (d), 121.9 (d), 125.1 (d), 125.5 (d), 125.5 (s), 128.6 (s), 130.8 (d), 132.0 (s), 134.0 (s), 136.5 (s), 137.0 (s), 169.2 (s). Anal. ($C_{16}H_{13}N_2OCl$) C, H, N.

2-(2-Acetylamino-5-methylphenyl)indole (10e): yield 96%; mp 168 °C; IR 3381 (*NH*Ac), 3362 (NH), 1672 (CO) cm^{-1}; ^1H NMR δ 2.04 (3H, s, CH_3), 2.37 (3H, s, CH_3), 6.70 (1H, d, $J = 1.3$ Hz, H-3), 7.00 (1H, dt, $J = 7.4$ Hz, $J = 1.3$ Hz, H-5), 7.12 (1H, dt, $J = 7.4$ Hz, $J = 1.3$ Hz, H-6), 7.15 (1H, dd, $J = 7.7$ Hz, $J = 1.3$ Hz, H-4′), 7.40 (1H, d, $J = 1.3$ Hz, H-6′), 7.44 (1H, dd, $J = 7.4$ Hz, $J = 1.3$ Hz, H-7), 7.48 (1H, d, $J = 7.7$ Hz, H-3′), 7.56 (1H, dd, $J = 7.4$ Hz, $J = 1.3$ Hz, H-4), 9.34 (1H, s, *NH*Ac), 11.33 (1H, bs, NH); ^{13}C NMR δ 20.6 (q), 23.5 (q), 101.3 (d), 111.3 (d), 119.1 (d), 120.0 (d), 121.4 (d), 126.9 (d), 127.1 (s), 128.2 (d), 128.4 (s), 129.2 (d), 132.4 (s), 134.6 (s), 135.0 (s), 136.5 (s), 170.6 (s). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

2-(2-Acetylaminophenyl)-5-chloroindole (10f): yield 99%; mp 170−171 °C; IR 3366 (*NH*Ac), 3265 (NH), 1680 (CO) cm^{-1}; ^1H NMR δ 2.04 (3H, s, CH_3), 6.69 (1H, d, $J = 1.4$ Hz, H-3), 7.10 (1H, dd, $J = 8.4$ Hz, $J = 2.1$ Hz, H-6), 7.30 (2H, dt, $J = 7.1$ Hz, $J = 1.6$ Hz, H-4′ and H-5′), 7.42 (1H, d, $J = 8.4$ Hz, H-7), 7.60 (2H, dd, $J = 7.1$ Hz, $J = 1.6$ Hz, H-3′ and H-6′), 7.61 (1H, d, $J = 2.1$ Hz, H-4), 9.48 (1H, s, *NH*Ac), 11.55 (1H, bs, NH); ^{13}C NMR δ 22.0 (q), 101.1 (d), 112.8 (d), 119.2 (d), 121.3 (s), 121.3 (d), 123.7 (s), 125.5 (d), 126.8 (d), 128.1 (d), 129.1 (d), 129.6 (s), 135.1 (s), 135.2 (s), 136.8 (s), 168.8 (s). Anal. ($C_{16}H_{13}N_2OCl$) C, H, N.

Synthesis of Substituted 3-Nitrosoindoles 11a−f. To a solution of the acetylamino derivatives **10a−f** (6 mmol) in acetic acid (30 mL) was added sodium nitrite (0.41 g, 6 mmol) dissolved in the minimum amount of water dropwise at 0−5 °C with stirring. After 1 h, an orange precipitate was formed. The mixture was poured onto crushed ice; the solid precipitated was filtered off, air-dried, and recrystallized from ethanol.

2-(2-Acetylaminophenyl)-3-nitrosoindole (11a): yield 97%; mp 228−230 °C; IR 3120 (*NH*Ac), 2820 (very broad NH),

1645 (CO) cm^{-1}; ^1H NMR δ 2.15 (3H, s, CH_3), 7.20 (1H, dt, J = 7.6 Hz, J = 2.0 Hz, H-5), 7.37 (1H, dt, J = 7.6 Hz, J = 2.0 Hz, H-6), 7.50 (1H, dt, J = 7.5 Hz, J = 1.2 Hz, H-4′), 7.53 (1H, dt, J = 7.5, J = 1.2 Hz, H-5′), 7.63 (1H, dd, J = 7.6 Hz, J = 2.0 Hz, H-7), 8.17 (1H, dd, J = 7.6 Hz, J = 2.0 Hz, H-4), 8.34 (1H, dd, J = 7.5 Hz, J = 1.2 Hz, H-6′), 8.45 (1H, dd, J = 7.5 Hz, J = 1.2 Hz, H-3′), 11.85 (1H, s, *NH*Ac), 13.72 (1H, broad NH); ^{13}C NMR δ 24.8 (q), 119.2 (s), 120.1 (d), 120.6 (d) 122.5 (d), 126.6 (d), 127.6 (d), 131.2 (d), 131.3 (s), 131.4 (d), 132.5 (s), 132.5 (d), 139.1 (2×s), 154.4 (s), 168.4 (s). Anal. ($C_{16}H_{13}N_3O_2$) C, H, N.

2-(2-Acetylamino-5-chlorophenyl)-3-nitrosoindole (11b): yield 96%; mp 250 °C; IR 3120 (*NH*Ac), 2847 (very broad NH), 1668 (CO) cm^{-1}; ^1H NMR δ 2.14 (3H, s, CH_3), 7.37 (1H, dt, J = 6.8 Hz, J = 1.5 Hz, H-5), 7.51 (1H, dd, J = 8.1 Hz, J = 2.2 Hz, H-4′), 7.54 (1H, dt, J = 6.8 Hz, J = 1,5 Hz, H-6), 7.59 (1H, dd, J = 6.8 Hz, J = 1.5 Hz, H-7), 8.13 (1H, dd, J = 6.8 Hz, J = 1.5 Hz, H-4), 8.42 (1H, d, J = 2.2 Hz, H-6′), 8.49 (1H, d, J = 8.1 Hz, H-3′), 12.05 (1H, s, NH), 14,13 (1H, broad NH); ^{13}C NMR δ 24.9 (q), 129.2 (s), 120.5 (d), 121.8 (s), 121.8 (d), 126.2 (s), 126.7 (d), 128.0 (d), 130.8 (d), 131.4 (d), 131.6 (d), 131.6 (s), 134.6 (s), 138.3 (s), 154.3 (s), 168.6 (s). Anal. ($C_{16}H_{12}N_3O_2$-Cl) C, H, N.

2-(2-Acetylaminophenyl)-5-methoxy-3-nitrosoindole (11c): yield 95%; mp 250 °C; IR 3120 (*NH*Ac), 2839 (very broad NH), 1626 (CO) cm^{-1}; ^1H NMR δ 1.91 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 7.10 (1H, dd, J = 7.1 Hz, J = 1.5 Hz, H-6), 7.18 (1H, dt, J = 8.5 Hz, J = 2.1 Hz, H-5′), 7.47 (1H, dt, J = 8.5 Hz, J = 2.1 Hz, H-4′), 7.55 (1H, d, J = 7.1 Hz, H-7), 7.73 (1H, d, J = 1.5 Hz, H-4), 8.32 (1H, dd, J = 8.5 Hz, J = 2.1 Hz, H-6′), 8.44 (1H, dd, J = 8.5 Hz, J = 2.1 Hz, H-3′), 11.90 (1H, s, NH), 12.45 (1H, broad NH); ^{13}C NMR δ 21.0 (q), 55.7 (q), 113.0 (d), 114.6 (d), 116.2 (d), 118.4 (s), 120.4 (d), 121.1 (s), 121.2 (s), 122.5 (d), 130.9 (d), 132.2 (d), 138.9 (s), 145.9 (s), 154.4 (s), 159.1 (s), 168.5 (s). Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

2-(2-Acetylamino-4-chlorophenyl)-3-nitrosoindole (11d): yield 95%; mp 232 °C; IR 3146 (*NH*Ac), 2859 (very broad NH), 1618 (CO) cm^{-1}; ^1H NMR δ 2.17 (3H, s, CH_3), 7.23 (1H, dd, J = 8.7 Hz, J = 2.2 Hz, H-5′), 7.37 (1H, dt, J = 7.4 Hz, J = 1.2 Hz, H-5), 7.51 (1H, dt, J = 7.4 Hz, J = 1.2 Hz, H-6), 7.58 (1H, dd, J = 7.4 Hz, J = 1.2 Hz, H-7), 8.14 (1H, dd, J = 7.4 Hz, J = 1.2 Hz, H-4), 8.43 (1H, d, J = 8.7 Hz, H-6′), 8.59 (1H, d, J = 2.2 Hz, H-3′), 12.28 (1H, s, *NH*Ac), 14.30 (1H, broad NH); ^{13}C NMR δ 25.2 (q), 117.5 (s), 119.6 (d), 120.1 (s), 120.6 (d), 122.5 (d), 127.0 (s), 127.0 (d), 128.1 (d), 131.8 (d), 134.0 (d), 136.3 (s), 140.8 (s), 140.9 (s), 154.7 (s), 169.2 (s). Anal. ($C_{16}H_{12}N_3O_2Cl$) C, H, N.

2-(2-Acetylamino-5-methylphenyl)-3-nitrosoindole (11e): yield 96%; mp 246 °C; IR 3131 (*NH*Ac), 2854 (NH), 1636 (CO) cm^{-1}; ^1H NMR δ 2.12 (3H, s, CH_3), 2.31 (3H, s, CH_3), 7.31 (1H, dd, J = 8.1 Hz, J = 1.4 Hz, H-4′), 7.36 (1H, dt, J = 7.2 Hz, J = 1.2 Hz, H-5), 7.52 (1H, dt, J = 7.2 Hz, J = 1.2 Hz, H-6), 7.60 (1H, dd, J = 7.2 Hz, J = 1.2 Hz, H-7), 8.13 (1H, d, J = 1.4 Hz, H-6′), 8.16 (1H, dd, J = 7.2 Hz, J = 1.2 Hz, H-4), 8.32 (1H, d, J = 8.1 Hz, H-3′), 11.70 (1H, s, *NH*Ac), 14.01 (1H, broad NH); ^{13}C NMR δ 20.5 (q), 24.8 (q), 119.3 (s), 120.2 (s), 120.6 (s), 120.8 (s), 126.7 (s), 127.6 (d), 127.8 (d), 131.4 (d), 131.8 (d), 131.8 (d), 132.5 (d), 132.6 (d), 136.9 (s), 154.5 (s), 168.2 (s). Anal. ($C_{17}H_{15}N_3O_2$) C, H, N.

2-(2-Acetylaminophenyl)-5-chloro-3-nitrosoindole (11f): yield 93%; mp 255−257 °C; IR 3120 (*NH*Ac), 2795 (NH), 1649 (CO) cm^{-1}; ^1H NMR δ 2.10 (3H, s, CH_3), 7.16 (1H, dt, J = 7.8 Hz, J = 1.2 Hz, H-4′), 7.48 (1H, dt, J = 7.8 Hz, J = 1.2 Hz, H-5′), 7.50 (1H, dd, J = 8.5 Hz, J = 2.0 Hz, H-6), 7.56 (1H, d, J = 8.5 Hz, H-7), 8.05 (1H, d, J = 2.0 Hz, H-4), 8.25 (1H, dd, J = 7.8 Hz, J = 1.2 Hz, H-6′), 8.38 (1H, dd, J = 7.8 Hz, J = 1.2 Hz, H-3′), 11.68 (1H, s, *NH*Ac), 14.26 (1H, broad NH); ^{13}C NMR δ 24.8 (q), 119.1 (s), 120.7 (d), 121.1 (d), 122.6 (d), 125.9 (d), 126.2 (s), 130.8 (d), 131.5 (d), 131.6 (2×s), 132.5 (d), 139.2 (2×s), 153.8 (s), 168.5 (s). Anal. ($C_{16}H_{12}N_3O_2Cl$) C, H, N.

Synthesis of Substituted Indolo[1,2-*c*]benzo[1,2,3]-triazines 5a−g. Hydrolysis of Compounds 11a−f. To a solution of acetylamino derivatives **11a−f** (1.6 mmol) in ethanol (10 mL) was added an aqueous solution of potassium hydroxide (15%, 10 mL). The reaction mixture was refluxed for 2 h. After cooling, the solution was neutralized with hydrochloric acid (1 N). The solid was filtered off, air-dried, and recrystallized from ethanol.

2-(2-Aminophenyl)-3-nitrosoindole (12a): yield 90%; mp 205 °C; IR 3351 and 3241 (NH_2), 3167 (NH) cm^{-1}; ^1H NMR δ 6.61 (1H, dt, J = 8.1 Hz, J = 1.9 Hz, H-5′), 6.87 (1H, dd, J = 8.1 Hz, J = 1.9 Hz, H-3′), 7.22 (1H, dt, J = 8.1 Hz, J = 1.9 Hz, H-4′), 7.30 (1H, dt, J = 7.4 Hz, J = 1.5 Hz, H-5), 7.50 (1H, dt, J = 7.4 Hz, J = 1.5 Hz, H-6), 7.53 (1H, dd, J = 7.4 Hz, J = 1.5 Hz, H-7), 7.63 (2H, s, NH_2), 8.19 (1H, dd, J = 7.4 Hz, J = 1.5 Hz, H-4), 8.48 (1H, dd, J = 8.1, Hz, J = 1.9 Hz, H-6′), 13.75 (1H, bs, NH); ^{13}C NMR δ 112.8 (s), 115.0 (d), 116.2 (d), 119.8 (d), 120.6 (s), 126.7 (d), 127.3 (d), 131.7 (d), 132.0 (d), 132.4 (d), 150.9 (s), 154.2 (s), 155.7 (s), 165.0 (s). Anal. ($C_{14}H_{11}N_3O$) C, H, N.

2-(2-Amino-5-chlorophenyl)-3-nitrosoindole (12b): yield 94%; mp 217 °C; IR 3321 and 3239 (NH_2), 3219 (NH) cm^{-1}; ^1H NMR δ 6.87 (1H, d, J = 8.9 Hz, H-3′), 7.20 (1H, dd, J = 8.9 Hz, J = 2.2 Hz, H-4′), 7.29 (1H, dt, J = 7.0 Hz, J = 1.7 Hz, H-5), 7.47 (1H, dt, J = 7.0 Hz, J = 1.7 Hz, H-6), 7.50 (1H, dd, J = 7.0 Hz, J = 1.7 Hz, H-7), 7.79 (2H, bs, NH_2), 8.14 (1H, dd, J = 7.0 Hz, J = 1.7 Hz, H-4), 8.53 (1H, d, J = 2.2 Hz, H-6′), 13.87 (1H, bs, NH); ^{13}C NMR δ 113.4 (s), 118.0 (d), 118.5 (s), 120.2 (d), 120.6 (s), 127.3 (d), 127.4 (d), 130.9 (d), 131.5 (d), 132.2 (d), 149.5 (s), 153.9 (s), 155.5 (s), 163.7 (s). Anal. ($C_{14}H_{10}N_3OCl$) C, H, N.

2-(2-Aminophenyl)-5-methoxy-3-nitrosoindole (12c): yield 90%; mp 206 °C; IR 3337 and 3231 (NH_2), 3148 (NH) cm^{-1}; ^1H NMR δ 3.84 (3H, s, CH_3), 6.61 (1H, dt, J = 7.5 Hz, J = 1.6 Hz, H-5′), 6.85 (1H, dd, J = 7.5 Hz, J = 1.6 Hz, H-3′), 7.06 (1H, dd, J = 8.4 Hz, J = 2.4 Hz, H-6), 7.19 (1H, dt, J = 7.5 Hz, J = 1.6 Hz, H-4′), 7.44 (1H, d, J = 8.4 Hz, H-7), 7.49 (2H, bs, NH_2), 7.75 (1H, d, J = 2.4 Hz, H-4), 8.42 (1H, dd, J = 7.5 Hz, J = 1.6 Hz, H-6′), 13.58 (1H, bs, NH); ^{13}C NMR δ 56.0 (q), 113.3 (s), 113.7 (d), 115.2 (d), 116.3 (d), 116.6 (d), 120.6 (d), 121.7 (s), 131.5 (d), 132.2 (d), 147.8 (s), 150.5 (s), 155.9 (s), 158.7 (s), 163.5 (s). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

2-(2-Amino-4-chlorophenyl)-3-nitrosoindole (12d): yield 90%; mp 243 °C; IR 3393 and 3261 (NH_2), 3132 (NH) cm^{-1}; ^1H NMR δ 6.64 (1H, dd, J = 8.3 Hz, J = 2.2 Hz, H-5′), 6.97 (1H, d, J = 2.2 Hz, H-3′), 7.29 (1H, dt, J = 7.2 Hz, J = 1.7 Hz, H-5), 7.48 (1H, dt, J = 7.2 Hz, J = 1.7 Hz, H-6), 7.53 (1H, dd, J = 7.2 Hz, J = 1.7 Hz, H-7), 7.90 (2H, s, NH_2), 8.19 (1H, dd, J = 7.2 Hz, J = 1.7 Hz, H-4), 8.52 (1H, d, J = 8.3 Hz, H-6′), 13.89 (1H, bs, NH); ^{13}C NMR δ 111.7 (s), 115.0 (d), 115.0 (d), 120.0 (d), 120.6 (s), 126.9 (d), 127.3 (d), 131.9 (s), 134.0 (d), 136.5 (d), 151.9 (s), 153.9 (s), 155.5 (s), 164.2 (s). Anal. ($C_{14}H_{10}N_3OCl$) C, H, N.

2-(2-Amino-5-methylphenyl)-3-nitrosoindole (12e): yield 95%; mp 190−191 °C; IR 3344 and 3213 (NH_2), 3120 (NH) cm^{-1}; ^1H NMR δ 2.20 (3H, s, CH_3), 6.76 (1H, d, J = 8.3 Hz, H-3′), 7.02 (1H, dd, J = 8.3 Hz, J = 2.2 Hz, H-4′), 7.26 (1H, dt, J = 7.3 Hz, J = 1.4 Hz, H-5), 7.40 (2H, bs, NH_2), 7.45 (1H, dt, J = 7.3 Hz, J = 1.4 Hz, H-6), 7.49 (1H, dd, J = 7.3 Hz, J = 1.6 Hz, H-7), 8.14 (1H, dd, J = 7.3 Hz, 1.4 Hz, H-4), 8.26 (1H, d, J = 2.2 Hz, H-6′), 13.74 (1H, bs, NH); ^{13}C NMR δ 20.3 (q), 112.6 (s), 116.2 (d), 119.5 (d), 120.4 (s), 122.9 (s), 126.4 (d), 127.1 (d), 131.7 (d), 131.9 (d), 132.7 (d), 140.0 (s), 148.6 (s), 155.6 (s), 162.6 (s). Anal. ($C_{15}H_{13}N_3O$) C, H, N.

2-(2-Aminophenyl)-5-chloro-3-nitrosoindole (12f): yield 95%; mp 228−230 °C; IR 3395 and 3231 (NH_2), 3123 (NH) cm^{-1}; ^1H NMR δ 6.59 (1H, dt, J = 7.4 Hz, J = 1.4 Hz, H-5′), 6.85 (1H, dd, J = 7.4 Hz, J = 1.4 Hz, H-3′), 7.19 (1H, dt, J = 7.4 Hz, J = 1.4 Hz, H-4′), 7.48 (1H, d, J = 7.0 Hz, H-7), 7.53 (1H, dd, J = 7.0 Hz, J = 1.4 Hz, H-6), 7.63 (2H, bs, NH_2), 8.12 (1H, d, J = 1.4 Hz, H-4), 8.43 (1H, dd, J = 7.4 Hz, J = 1.4 Hz, H-6′), 14.01 (1H, bs, NH); ^{13}C NMR δ 112.4 (s), 112.9 (d), 116.2 (d), 120.8 (d), 121.6 (s), 126.5 (d), 130.3 (s), 131.2 (d), 131.8 (d), 132.1 (d), 150.8 (s), 152.5 (s), 154.8 (s), 165.0 (s). Anal. ($C_{14}H_{10}N_3OCl$) C, H, N.

Diazotization of Substituted 2-(2-Aminophenyl)-3-nitrosoindoles 12a−f. Compounds **12a−f** were diazotized following the procedure described for compound **9**. The solid

precipitated was filtered off, air-dried, and pure enough to give satisfactory analytical and spectral data.

12-Nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5a): yield 80%; mp 210 °C; IR 1471 (NO), 1448 (N═N) cm^{-1}; ^1H NMR δ 7.77 (1H, dt, J = 7.3 Hz, J = 1.5 Hz, H-10), 7.82 (1H, dt, J = 7.3 Hz, 1.5 Hz, H-9), 8.35 (1H, dt, J = 8.4 Hz, J = 1.9 Hz, H-3), 8.37 (1H, dt, J = 8.4 Hz, J = 1.9 Hz, H-2), 8.43 (1H, dd, J = 7.3 Hz, J = 1.5 Hz, H-11), 8.56 (1H, dd, J = 8.4 Hz, J = 1.9 Hz, H-4), 8.75 (1H, dd, J = 7.3 Hz, J = 1.5 Hz, H-8), 9.46 (1H, dd, J = 8.4 Hz, J = 1.9 Hz, H-1). Anal. ($C_{14}H_8N_4O$) C, H, N.

2-Chloro-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5b): yield 80%; mp 207 °C; IR 1466 (NO), 1448 (N═N) cm^{-1}; ^1H NMR δ 7.75 (2H, dt, J = 7.7 Hz, J = 2.0 Hz, H-9 and H-10), 8.32 (1H, dd, J = 7.7 Hz, J = 2.0 Hz, H-8), 8.35 (1H, dd, J = 7.7 Hz, J = 2.0 Hz, H-11), 8.49 (1H, dd, J = 8.8 Hz, J = 2.3 Hz, H-3), 8.70 (1H, d, J = 8.8 Hz, H-4), 9.25 (1H, d, J = 2.3 Hz, H-1). Anal. ($C_{14}H_7N_4OCl$) C, H, N.

10-Methoxy-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5c): yield 85%; mp 208 °C; IR 1473 (NO), 1458 (N═N) cm^{-1}; ^1H NMR δ 7.36 (1H, dd, J = 9.0 Hz, J = 2.4 Hz, H-9), 7.93 (1H, d, J = 2.4 Hz, H-11), 8.33 (2H, dt, J = 6.8 Hz, J = 1.4 Hz, H-2 and H-3), 8.45 (1H, d, J = 9.0 Hz, H-8), 8.71 (1H, dd, J = 6.8 Hz, J = 1.4 Hz, H-4), 9.40 (1H, dd, J = 6.8 Hz, J = 1.4 Hz, H-1). Anal. ($C_{15}H_{10}N_4O_2$) C, H, N.

3-Chloro-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5d): yield 83%; mp 207 °C; IR 1464 (NO), 1446 (N═N) cm^{-1}; ^1H NMR δ 7.79 (1H, dt, J = 7.3 Hz, J = 1.6 Hz, H-10), 7.82 (1H, dt, J = 7.3 Hz, J = 1.6 Hz, H-9), 8.39 (1H, dd, J = 7.3 Hz, J = 1.6 Hz, H-11), 8.41 (1H, dd, J = 7.3 Hz, J = 1.6 Hz, H-8), 8.55 (1H, dd, J = 8.7 Hz, J = 2.1 Hz, H-2), 8.91 (1H, d, J = 2.1 Hz, H-4), 9.41 (1H, d, J = 8.7, H-1). Anal. ($C_{14}H_7N_4OCl$) C, H, N.

2-Methyl-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5e): yield 100%; mp 190−191 °C; IR 1479 (NO), 1448 (N═N) cm^{-1}; ^1H NMR δ 2.70 (3H, s, CH_3), 7.73 (2H, dt, J = 7.4 Hz, J = 1.5 Hz, H-9 and H-10), 8.15 (1H, dd, J = 8.8 Hz, J = 2.5 Hz, H-3), 8.36 (1H, dd, J = 7.4 Hz, J = 1.5 Hz, H-8), 8.47 (1H, dd, J = 7.4 Hz, J = 1.5 Hz, H-11), 8.57 (1H, d, J = 8.8 Hz, H-4), 9.18 (1H, d, J = 2.5 Hz, H-1). Anal. ($C_{15}H_{10}N_4O$) C, H, N.

10-Chloro-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5f): yield 100%; mp 210 °C; IR 1475 (NO), 1448 (N═N) cm^{-1}; ^1H NMR δ 7.76 (1H, dd, J = 8.8 Hz, J = 2.0 Hz, H-9), 8.31 (2H, dt, J = 7.8 Hz, J = 1.6 Hz, H-2 and H-3), 8.34 (1H, d, J = 2.0 Hz, H-11), 8.53 (1H, d, J = 8.8 Hz, H-8), 8.70 (1H, dd, J = 7.8 Hz, J = 1.6 Hz, H-4), 9.36 (1H, dd, J = 7.8 Hz, J = 1.6 Hz, H-1). Anal. ($C_{14}H_7N_4OCl$) C, H, N.

10-Nitro-12-nitrosoindolo[1,2-*c*]benzo[1,2,3]triazine (5g). This compound was prepared by nitration of the unsubstituted indolobenzotriazine **5a**. Thus to a solution of **5a** (0.5 g, 2 mmol) in sulfuric acid (96%, 12 mL) was added potassium nitrate (0.2 g, 2 mmol) dissolved in sulfuric acid (96%, 5 mL) at 0 °C dropwise with stirring. The reaction mixture was allowed to reach room temperature, kept under stirring overnight, and poured onto crushed ice. The solid precipitate was filtered off, air-dried, and pure enough to give satisfactory analytical and spectral data: yield 60%; mp 215 °C; IR 1525 (NO_2), 1494 (NO), 1455 (N═N) cm^{-1}; ^1H NMR δ 8.30 (1H, dt, J = 7.4 Hz, J = 1.8 Hz, H-3), 8.39 (1H, dt, J = 7.4 Hz, J = 1.8 Hz, H-2), 8.53 (1H, dd, J = 8.9 Hz, J = 2.4 Hz, H-9), 8.73 (1H, d, J = 8.9 Hz, H-8), 8.75 (1H, dd, J = 7.4 Hz, J = 1.8 Hz, H-4), 9.02 (1H, d, J = 2.4 Hz, H-11), 9.43 (1H, dd, J = 7.4 Hz, J = 1.8 Hz, H-1). Anal. ($C_{14}H_7N_5O_3$) C, H, N.

(B) Biology. Compounds. Test compounds were dissolved in DMSO at an initial concentration of 200 μM and then were serially diluted in culture medium.

Cells. Cell lines were from American Type Culture Collection (ATCC). The human nasopharyngeal carcinoma KB cell line and the drug-resistant subclones KB^{MDR}, KB^{V20C}, KB^{70}, and KB^{Camp} were a generous gift of Prof. Y. C. Cheng, Yale University. Bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains from ATCC.

$H9/III_B$, MT-4, and C8166 (grown in RPMI 1640 containing 10% fetal calf serum (FCS), 100 U/mL penicillin G, and 100 μg/mL streptomycin) cells were used for anti-HIV assays. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect kit (Gibco).

Viruses. Human immunodefieciency virus type-1 (HIV-1, III_B strain) was obtained from supernatants of persistently infected $H9/III_B$ cells. HIV-1 stock solutions had a titer of 5×10^7 cell culture infectious dose fifty ($CCID_{50}$)/mL.

Antiviral Assays. Activity of compounds against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 μL of RPMI 10% FCS containing 1×10^4 cells was added to each well of flat-bottomed microtiter trays containing 50 μL of medium and serial dilution of test compounds; 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ was added. After a 4-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[9,10] Cytotoxicity of compounds, based on the viability of mock-infected cells, as monitored by the MTT method, was evaluated in parallel with their antiviral activity.

Antitumor Assays. Exponentially growing leukemia and lymphoma cells were resuspended at a density of 1×10^5 cells/mL in RPMI containing serial dilutions of the test drugs. Cell viability was determined after 4 days at 37 °C by the MTT method. Activity against cell lines derived from solid tumors was evaluated in exponentially growing cultures seeded at 5×10^4 cells/mL which were allowed to adhere for 16 h to culture plates before addition of the drugs. Cell viability was determined by the MTT method 4 days later.

Linear Regression Analysis. Tumor cell growth at each drug concentration was expressed as percentage of untreated controls, and the concentration resulting in 50% (EC_{50}, IC_{50}) growth inhibition was determined by linear regression analysis.

Cytotoxicity Assays. Peripheral blood lymphocytes (PBL) from HIV-negative donors were obtained by separation on Fycoll-Hypaque gradients. After extensive washings, cells were resuspended (1×10^6 cells/mL) in RPMI-1640 with 10% FCS and incubated overnight.

For cytotoxicity evaluations in proliferating PBL cultures, nonadherent cells were resuspended at 1×10^6 cells/mL in growth medium, stimulated with PHA (2.5 μg/mL) for 24 h before dilution to 1×10^5 cells/mL in medium containing PHA (2.5 μg/mL), IL-2 (50 U/mL), and various concentrations of the test compounds. Viable cell number was determined 6 days later. Under these conditions, untreated PBL were able to undergo exponential growth up to four cell cycles, as determined by viable cell counts.

For cytotoxicity evaluations in resting PBL cultures, nonadherent cells were resuspended at high density (1×10^6 cells/mL) and treated for as long as 3 days with the test compounds. Then, the cells were extensively washed to remove the inhibitors and were stimulated with PHA for 24 h before being diluted to 1×10^5 cells/mL in medium containing PHA and IL-2. Cell viability was determined after incubation at 37 °C for 6 days.

Antibacterial Assays. *S. aureus*, group D *Streptococcus*, *Salmonella spp.*, and *Shigella spp.* were recent clinical isolates. Assays were carried out in nutrient broth, pH 7.2, with an inoculum at 10^3 bacterial cells/tube. Minimum inhibitory concentrations (MIC) were determined after incubation at 37 °C for 18 h in the presence of serial dilutions of the test compounds.

Antimycotic Assays. Yeast inocula were obtained by properly diluting cultures incubated at 37 °C for 30 h in Sabouraud dextrose broth to obtain 5×10^3 cells/mL. On the contrary, dermatophyte inocula were obtained from cultures grown at 37 °C for 5 days in Sabouraud dextrose broth by finely dispersing clumps with a glass homogenizer before diluting to 0.05 OD_{590}/mL. Then, 20 μL of the above suspensions was added to each well of flat-bottomed microtiter trays containing 80 μL of medium with serial dilutions of test

compounds, and the trays were incubated at 37 °C. Growth controls were visually determined after 2 days (yeast) or 3 days (dermatophytes).

MIC was defined as the compound concentration at which no macroscopic signs of fungal growth were detected. The minimal germicidal concentration (MBC or MFC) was determined by subcultivating in Sabouraud dextrose agar samples from cultures with no apparent growth.

Antimycobacterial Assays. *M. smegmatis*, *M. avium* complex (MAC), and *M. tuberculosis* 27294 were ATCC strains, whereas *M. fortuitum* and *M. tuberculosis* 1104 were clinical isolates. MICs were assessed in microtiter plates by adding 20-μL aliquots of a *Mycobacterium* suspension (whose turbidity was equal to that of a 1 McFarland standard containing 10^8 CFU/mL) to 80 μL of Middlebrood 7II9 medium containing 0.5% glicerol and 10% OADC and various concentrations of the test compounds. The plates were then incubated for 1 day (*M. smegmatis* and *M. fortuitum*) or 9 days (MAC and *M. tuberculosis*) at 37 °C. At the end of incubation, the number of viable *Mycobacteria* was determined by the MTT method.

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